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Structure of mixed β-lactoglobulin/pectin adsorbed layers at air/water interfaces; a spectroscopy study

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Abstract

Based on earlier reported surface rheological behaviour two factors appeared to be important for the functional behaviour of mixed protein/polysaccharide adsorbed layers at air/water interfaces: (1) protein/polysaccharide mixing ratio and (2) formation history of the layers. In this study complexes of β-lactoglobulin (positively charged at pH 4.5) and low methoxyl pectin (negatively charged) were formed at two mixing ratios, resulting in negatively charged and nearly neutral complexes. Neutron reflection showed that adsorption of negative complexes leads to more diffuse layers at the air/water interface than adsorption of neutral complexes. Besides (simultaneous) adsorption of protein/polysaccharide complexes, a mixed layer can also be formed by adsorption of (protein/)polysaccharide (complexes) to a pre-formed protein layer (sequential adsorption). Despite similar bulk concentrations, adsorbed layer density profiles of simultaneously and sequentially formed layers were persistently different, as illustrated by neutron reflection analysis. Time resolved fluorescence anisotropy showed that the mobility of protein molecules at an air/water interface is hampered by the presence of pectin. This hampered mobility of protein through a complex layer could account for differences observed in density profiles of simultaneously and sequentially formed layers. These insights substantiated the previously proposed organisations of the different adsorbed layers based on surface rheological data.

Keywords: Protein; Polysaccharide; Adsorption; Neutron reflection; Fluorescence anisotropy

1. Introduction

A combination of proteins and polysaccharides is often used for stabilisation of foams and emulsions, for instance in food- or pharmaceutical applications. Non-surface-active anionic polysaccharides like pectin, carrageenan, and dextran sulfate have been reported to affect surface rheological properties by interaction with protein adsorbed at interfaces [1,2]. The net charge and the thickness of adsorbed layers are believed to be affected by co-adsorption of anionic polysaccharides [3,4]. An increased net charge and an increased layer thickness at an oil/water interface provides electrostatic and steric repulsion between emulsion droplets respectively [5–8], providing increased emulsion stability. Alternatively, foam stability may be enhanced by similar effects taking place at the air/water interface [9]. In a recent paper [10] surface rheological measurements on several protein and mixed protein/polysaccharide adsorbed layers at air/water interfaces have been discussed. On the basis of these data the following hypothetical organisations of the various layers was proposed as depicted in Fig. 1.

Two factors determine the functional behaviour of the adsorbed layers: (i) the net charge of the protein/polysaccharide complexes and thus the stoichiometry of these two biopoly-

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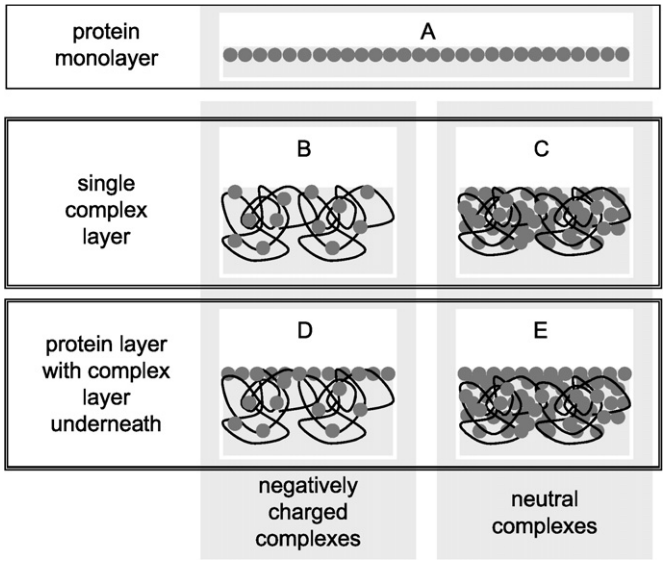

Fig. 1. Schematic representation of different adsorbed layers at the air/water interface: (A) protein monolayer, (B) and (C) mixed layers from simultaneous adsorption where (B) is from negatively charged complexes and (C) from net neutral complexes, (D) and (E) complexes adsorbed at previously formed protein layer where (D) are negatively charged complexes and (E) net neutral complexes.

mers, and (ii) the formation history of the layers. Negatively charged protein/polysaccharide complexes form adsorbed layers (Fig. 1B) with lower dilatational modulus than a pure protein layer (Fig. 1A). It was suggested that the presence of an excess of anionic polysaccharide is able to prevent the formation of a dense protein layer at the air/water interface. A layer formed from neutral protein polysaccharide complexes had a similar dilatational modulus as a pure protein layer, while its surface shear modulus was much higher than that of the pure protein layer. This indicated that dense and thick layers may be formed from neutral complexes (Fig. 1C). When mixed layers were prepared in an alternative way, by sequential adsorption, a dense adsorbed protein layer could be formed before the polysaccharide was introduced: adsorption of the polysaccharide, or protein/polysaccharide complexes, to a previously formed protein layer increased both the dilatational and surface shear modulus (Figs. 1D and 1E). Although final protein and polysaccharide concentrations in the bulk solution were the same in Figs. 1B and 1D, surface rheological behaviour suggested that even after 20 h, the density of layer B was still lower than that of layer D. This history dependent behaviour suggested that the layer structures may not quickly reorganise to a thermodynamically equilibrium situation. This model is, however, based on interpretation of system properties and thus a more molecular substantiation is lacking.

Specular neutron reflectivity may give insight in the structure (layer thickness and density profile) of adsorbed layers at air/water interfaces by providing scattering length density profiles normal to the interface at Angstrom resolution. By varying the H_2O/D_2O ratio the contrast between protein and solvent can be changed, allowing one to perform simultaneous fitting of measurements performed on the same protein(/polysaccharide) system at different contrasts yielding more reliable fit results. In this way this technique can provide detailed density profiles of interface layers [11].

Time resolved fluorescence anisotropy (TRFA) has proven to be a useful tool to probe rotational mobility of protein molecules at an air/water interface [12]. With this technique fluorescently-labelled protein molecules (either free in solution or complexed with polysaccharide) are adsorbed to an air/water interface. An excitation pulse of polarised light is aimed at the interface. Labels parallel to the polarisation angle are preferentially excited. After the excitation pulse, the anisotropy (which is—in 2D experiments as these—equal to the polarisation) of emitted light was followed during the lifetime of the label. Emitted light is polarised unless the labels (or the labelled protein molecules) have reoriented before emission takes place. Hence, anisotropy decay in time gives information about rotational mobility of (individual segments of) protein molecules. Changes in protein mobility at the interface as a result of the presence of polysaccharide may provide insight in the molecular interactions and thus the structural organisation.

The combination of thicknesses and density profiles of the different layers to be obtained by neutron reflection and the mobility of protein molecules in these layers to be determined by TRFA may elucidate the molecular structure of mixed protein/polysaccharide adsorbed layers at air/water interfaces. The aim of this work is to validate the hypothesised mixed layer structures (as shown in Fig. 1), depending on protein/polysaccharide mixing ratio and the sequence of adsorption to the air/water interface.

2. Materials and methods

2.1. Materials

Bovine β-lactoglobulin was purified using a non-denaturing method as described previously [13]. Low methoxyl pectin was supplied by CP Kelco (Lille Skensved, Denmark). The degree of methylation is 30.4% (the non-methylated galacturonic acid monomers possess a free carboxyl group) and the uronic acid content is 78.5% [14]. The average molar mass (M_n) is 1.5×10^5 g/mol, the polydispersity (M_w/M_n) 2.4 [15] and the pK_a ~4.5 (based on proton titration curves, not shown). Pectin solutions in H_2O (deionised water, Barnstead EASYpure UV, USA) and in D_2O (99 atom%, Sigma-Aldrich) were prepared by slowly dispersing the powder on the surface of the thoroughly stirred (magnetic stirrer) and heated (~50 °C) H_2O/D_2O and subsequently heating at 70 °C for 30 min. After overnight storage at 35 °C, the samples were centrifuged at 6000*g* for 10 min and stored at 4 °C until further use. Solubility of pectin in H_2O and D_2O did not differ more than 10% as measured by an automated colourimetry *m*-hydroxydiphenyl method [16,17]. For the samples in Null Reflecting Water (NRW) stock solutions in H_2O and D_2O were mixed to 8.73% w/w (corresponding to NRW at 22 °C) D_2O in H_2O and stored overnight at 4 °C to equilibrate H/D exchange ratio between pectin and solvent. Protein stock solutions were prepared by dissolving the freeze dried material in D_2O and NRW (pH after dissolution ~6.8) and storing overnight to equilibrate H/D exchange ratio be-